Global Change Biology (2002) **8,** 710–721

Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (*Phaseolus vulgaris* L.)

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Abstract

It is important to quantify and understand the consequences of elevated temperature and carbon dioxide (CO_2) on reproductive processes and yield to develop suitable agronomic or genetic management for future climates. The objectives of this research work were (a) to quantify the effects of elevated temperature and CO_2 on photosynthesis, pollen production, pollen viability, seed-set, seed number, seeds per pod, seed size, seed yield and dry matter production of kidney bean and (b) to determine if deleterious effects of high temperature on reproductive processes and yield could be compensated by enhanced photosynthesis at elevated CO_2 levels. Red kidney bean cv. Montcalm was grown in controlled environments at day/night temperatures ranging from 28/18 to 40/30 °C under ambient (350 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) CO_2 levels. There were strong negative relations between temperature over a range of 28/18–40/30 °C and seed-set (slope, −6.5% $°C^{-1}$) and seed number per pod (−0.34 $°C^{-1}$) under both ambient and elevated CO_2 levels. Exposure to temperature >28/18 °C also reduced photosynthesis (−0.3 and −0.9 μmol m^{-2} s^{-1} $°C^{-1}$), seed number (−2.3 and −3.3 $°C^{-1}$) and seed yield (−1.1 and −1.5 g $plant^{-1}$ $°C^{-1}$), at both the CO_2 levels (ambient and elevated, respectively). Reduced seed-set and seed number at high temperatures was primarily owing to decreased pollen production and pollen viability. Elevated CO_2 did not affect seed size but temperature >31/21 °C linearly reduced seed size by 0.07 g $°C^{-1}$. Elevated CO_2 increased photosynthesis and seed yield by approximately 50 and 24%, respectively. There was no beneficial interaction of CO_2 and temperature, and CO_2 enrichment did not offset the negative effects of high temperatures on reproductive processes and yield. In conclusion, even with beneficial effects of CO_2 enrichment, yield losses owing to high temperature (>34/24 °C) are likely to occur, particularly if high temperatures coincide with sensitive stages of reproductive development.

Keywords: climate change, elevated carbon dioxide, elevated temperature, Phaseolus bean, photosynthesis, seed-set

Received 20 July 2001; revised version received 9 January and accepted 25 January 2002

Introduction

There is currently considerable concern about the increasing carbon dioxide (CO_2) concentration in the atmosphere, associated increases in temperature and their effects on crop production. Atmospheric CO_2 concentration has risen from 280 to 370 μmol mol^{-1}, from preindustrial times to the current year (IPCC, 2001). This rising trend is expected to continue and result in an increase to nearly 700 μmol mol^{-1} by the end of this century if no steps are taken to limit the emission of CO_2 from various sources, particularly from fossil fuels (IPCC, 2001). The associated increase in the mean temperature is likely to be in the order of 1.5–4.2 °C because of rising greenhouse gases including CO_2, methane and nitrous oxide. Bean is one of the important leguminous crops cultivated as a source of vegetable protein over a range of climates from northern Europe and America to tropics

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of America, Asia and Africa (Davis, 1997). These changes in CO_2 level and temperature can have significant impact on growth, dry matter production and yield of bean crops.

Research has shown that bean is sensitive to high temperatures particularly during flower development, as high temperature results in reduced pod and seed-set (Monterroso & Wien, 1990; Konsens *et al*., 1991; Gross & Kigel, 1994). Gross & Kigel (1994) reported lowest pod-set when flower buds were exposed to high temperature (32/27 °C) 6–12 days prior to anthesis and at anthesis. Sensitivity to high temperature decreased as anthesis approached and postfertilization stages were more tolerant to high temperature than prefertilization stages (Gross & Kigel, 1994). Lower pod-set and lower seed-set at high temperature were owing to nonviable pollen, failure of anther dehiscence, reduced pollen tube penetration into the stigma and impaired female performance (Gross & Kigel, 1994). Controlled environmental studies showed that growth and dry matter production of bean were increased under elevated levels of CO_2 up to 1200 μmol mol^{-1} of CO_2 (Jolliffe & Ehret, 1985). Elevated CO_2 levels increase photosynthesis in many crop species (Boote *et al*., 1997), but it is not known if these high rates of photosynthesis can compensate for the loss caused by high temperature on reproductive growth and development of bean.

Under future climate change scenarios, it is most likely that plants will be exposed to a combination of both higher temperatures and CO_2 (Rosenzweig & Hillel, 1998). Therefore, it is important to understand the combined effects of elevated temperature and CO_2 for determining agricultural management or genetic improvement required to sustain bean productivity in future climates. Data on interaction effects of high temperature and elevated CO_2 are not available for bean. However, studies on cowpea (*Vigna unguiculata* L. Walp.; Ahmed *et al*., 1993) and soybean (*Glycine max* L. Merill; Baker *et al*., 1989) suggest that there was no beneficial interaction of CO_2 at higher temperatures. Thus, the objectives of this research work were (a) to quantify the interactive effects of elevated temperature and CO_2 on photosynthesis, pollen production, pollen viability, seed-set, seed numbers, seeds per pod, seed size, seed yield and dry matter production of red kidney bean and (b) to determine if deleterious effects of high temperature on reproductive processes and yield could be compensated by enhanced photosynthesis at elevated CO_2 levels.

Materials and methods

This research was conducted between August and November 2000, in naturally sunlit, controlled-environment chambers at the Plant and Soil Science Field Teaching Laboratory of the University of Florida in Gainesville (29°68′-N, 82°27′-W), USA.

Growth conditions

The experiment was conducted in sunlit, controlled-environment growth chambers. Each chamber has a 2-m wide, 1-m long and 1.5-m high canopy compartment made of clear 'Sixlight' plastic (Taiyo Kogyo Co., Tokyo) on aluminium frames fitted over a soil compartment 0.6-m deep. Plants were grown in eight chambers maintained at a cyclic day/night maximum/minimum temperature regime of 31/21 °C from sowing to emergence, i.e. 8 days after sowing (DAS). Thereafter, each chamber was used to impose one of the eight treatments: day/night maximum/minimum temperature regimes of 28/18, 31/21, 34/24, 37/27 and 40/30 °C at elevated (700 μmol mol^{-1}) and at 28/18, 34/24 and 40/30 °C at near-ambient (350 μmol mol^{-1}) CO_2 levels until maturity. The dew-point temperature was maintained 5 °C below the target day and night temperatures in each chamber, and was measured with dew point hygrometers (Dew-10, General Eastern Instrument, Woburn, MA). The dew-point and dry-bulb temperatures were controlled by a cold-water heat exchanger (Dunham-Bush, Harrisonburg, Virginia, USA) in conjunction with an electrical- resistance heater (AA Electric, Lakeland, Florida, USA), which removes the excess humidity and controls set point temperature. Air temperature in each chamber was controlled on a sinusoidal wave (from T_{min} at 06.00 h to T_{max} at 14.00 h with a decay function at night). The air temperatures were measured in each chamber at 1-m above the soil by using radiation shielded and aspirated copper-constantan thermocouples (Omega Engineering, Stamford, Conneticut, USA). Readings were taken every 1 s and means of successive 5-min periods were stored using a data logger (CR10, Campbell Scientific Inc, North Logan, Utah). Solar photosynthetic photon flux density (PPFD) was measured in chamber using calibrated photoelectric cells (Panasonic, Atlanta, Georgia). The chambers typically transmit about 87% of the incoming PAR.

Carbon dioxide concentration in each chamber was measured and maintained either at near ambient (350 μmol mol^{-1}) or elevated (700 μmol mol^{-1}) as per treatment by injecting CO_2 into the chambers from high-pressure 100% CO_2 cylinders. Mean and standard errors of actual daytime CO_2 concentrations for successive 5-min sampling periods across the entire season were 354 ± 1.2 μmol mol^{-1} at ambient and 696 ± 1.4 μmol mol^{-1} at elevated CO_2 treatments. Night-time CO_2 concentration was controlled to approximately ambient by automatically venting and flushing the chambers with ambient air once every hour during the night. The rise in CO_2 owing to respiration was also monitored.

The CO_2 concentrations were measured by infrared gas analyser (Siemens Corporation, New York, USA), and controlled and recorded by the CR10 data loggers. The details of the chamber characteristics, function of chambers, specific methods for controlling set chamber environments, and the quality of environmental control are described by Pickering *et al*. (1994).

Plant husbandry

Uniform seeds of red kidney bean cv. Montcalm were selected and sown (two per hill) at a depth of 3 cm in north–south rows at spacing of 33 × 10 cm (six, 0.9-m long rows per chamber and 9 hills per row) on 15 August 2000. Plants were irrigated through overhead sprinklers from sowing to 20 DAS and thereafter they were dependent on subsurface irrigation provided by a constant water table at 40–45 cm from the soil surface. This worked well as there was good capillary water rise in the Kendrick fine sand (a member of the loamy, siliceous, hyperthermic family of Arenic Paleudult). Thinning was done at 10 d after emergence leaving one plant per hill (i.e. nine plants per row). The crop was kept weed free and healthy throughout the season by hand weeding. The biological predator green lacewing (*Chysoperla* spp.) was released to prevent incidence of aphids (*Aphis fabae* Scopoli), white fly (*Bemisia tabaci* Gennadius) and red spider mites (*Tetranychus urticae* Koch). There were no pests or disease incidence and plants were healthy throughout the experiment.

The seeds were inoculated with *Rhizobium* (Nitrogin; Lipha Tech Inc, Milwaukee, Wisconsin, USA). Prior to sowing, the soil was fertilized with 60 g N, 60 g P and 60 g K m^{-2} as a basal application by broadcasting fertilizer in the soil and incorporating to 15-cm depth. At the same time organic nematicide, i.e. Nem-A-cide [a.i. Chitin (poly N-acetyl-D-glucosamine) protein; Voluntary Purchasing Groups Inc, Bonham, Texas, USA] was incorporated into the soil at 250 g m^{-2} to protect from nematodes. At 60 DAS plants were again fertilized with 40 g N, 40 g P and 40 g K m^{-2} with water-soluble fertilizer.

Seed-set and pollen studies

To follow the fate of individual flower buds, a total of 40 floral buds (not opened; green-white bud stage) were randomly selected in each treatment on 12–18 different plants, i.e. about 2–4 buds per plant. Floral buds were tagged on racemes spanning the height of the plant (terminal, middle and bottom), while within each raceme, buds located in the middle were selected, and extreme ends were avoided. Tagging was done on day 3 after first flowering. The ability of flowers to set pods (pod-set) and seeds (seed-set) was estimated 30 days later. Pod-set was defined as the proportion of the 40 tagged floral buds that set pod; whereas, seed-set was defined as the proportion of the 40 tagged floral buds that produced seed (expressed as percentage).

Six individual floral buds at green-white bud stage were collected between 08.00 and 09.00 h from each chamber for 3 successive days starting from the day of tagging. A total of 18 buds per treatment were selected from 18 different plants, and data on the number of pollen grains and pollen viability was measured for each bud. The number of pollen grains per flower were counted using a haemacytometer (Hausser Scientific, Horsham, PA) and pollen viability was measured by staining with 1% triphenyl tetrazolium chloride solution as described by Kearns & Inouye (1993). Anthers were collected from flower buds and were split open on a glass slide and stained. The pollen grains stained red were considered viable, whereas those that remained transparent were classified as dead. The numbers of viable and nonviable pollen grains were counted and proportion of viable pollen was estimated as the ratio of the number of viable pollen grains to the total number of pollen grains (expressed as percentage).

Photosynthesis measurements

Photosynthetic rates, stomatal conductance, and transpiration rates were measured on individual attached leaves at 35 DAS (when plants had attained a good canopy cover and near the time when tagged flower buds were setting pods) on a clear sunny day between 11.00 and 14.00 h using a LI-COR LI-6200 portable photosynthesis system (LI-COR, Lincoln, USA) with a 1-L leaf chamber. Each observation was repeated nine times on three randomly selected, fully expanded leaves of three different plants after the measuring cuvette had equilibrated to the temperature and CO_2 levels in the growth chamber and when the solar PPFD was saturating at 1200–1800 μmol m^{-2} s^{-1}. The duration of each measurement typically lasted 45 s. Leaflets from the photosynthesis measurements were harvested and their leaf area recorded. Leaf area was measured using the LI-3100 leaf area meter (LI-COR, Lincoln, USA) and photosynthetic rates were expressed on a leaf area basis.

Yield measurement

At maturity subsamples of six randomly selected plants (one from each row) were taken from each chamber and separated into component parts (leaves, stems and pods) and their respective dry weights were recorded after drying to constant weight at 60 °C. After drying, seeds

were separated from pod walls and data on number of seeds per pod, individual seed weight (seed size), number of seeds per plant, and seed yield per plant were recorded.

Data analysis

The data on pod-set, seed-set and pollen viability were in proportions (percentages) and therefore were subjected to angular transformation before statistical analysis. The effects of temperature and CO_2 on measured variables were statistically analysed by comparison of regression lines (Mead *et al*., 1993) using STATISTIX 7 for Windows package (Analytical Software, Tallahassee, FL).

Results

The time from sowing to first flower varied with temperature treatments, but there was no effect of CO_2 levels. The duration from sowing to first flower at day/night temperatures of 28/18, 31/21, 34/24, 37/27 and 40/30 °C was 30, 27, 28, 31 and 43 days, respectively, at both the CO_2 levels. The corresponding times from planting to maturity were 73, 70, 68, 70, and 71 days, respectively.

Pod and seed-set

There were no effects of temperature and/or CO_2 on the percentage of flowers setting pods (Fig. 1a) at temperatures between 28/18 and 37/27 °C for both ambient and

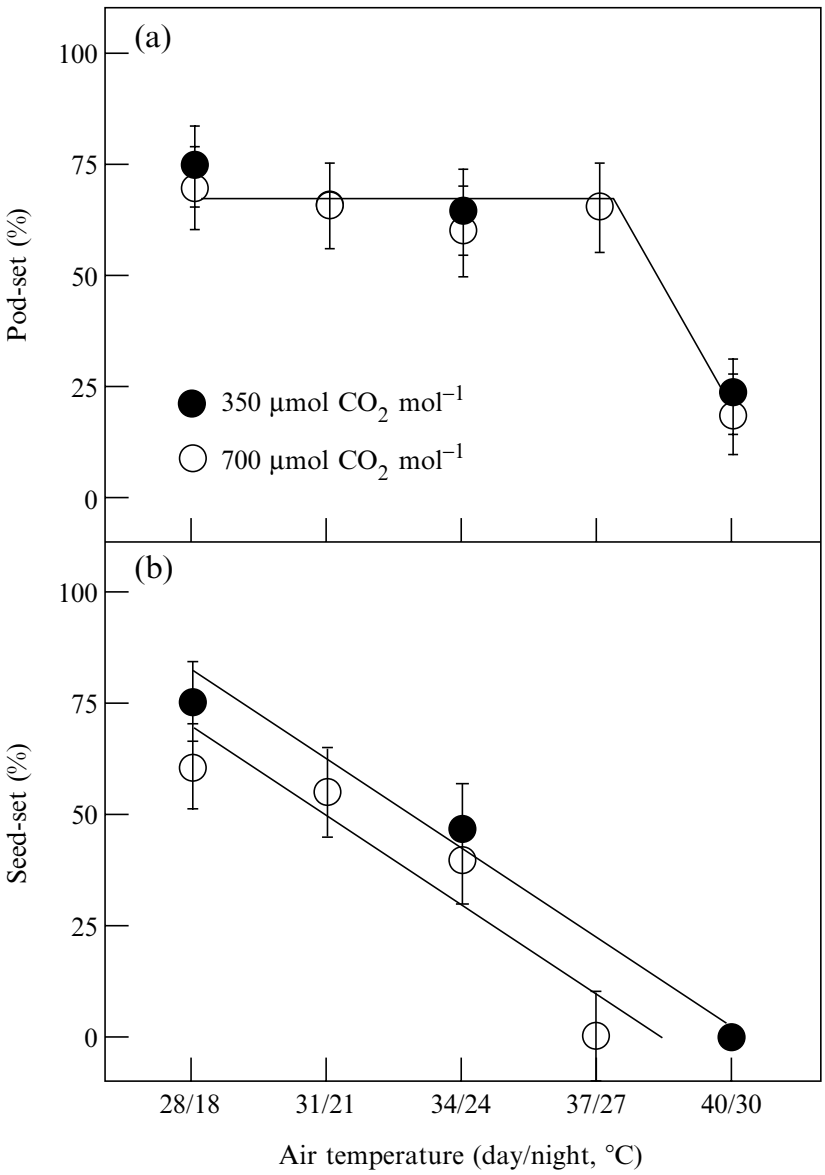

Fig. 1 Relations between day/night air temperature (°C) and (a) percentage of flowers setting pods (pod-set, angular transformed) and; (b) percentage of flowers setting seeds (seed-set, angular transformed) at ambient (●, 350 µmol mol^{-1}) and elevated (○, 700 µmol mol^{-1}) CO_2 levels. Fitted regressions for sloping lines in (a) y = 699 (± 49)−17 (± 1.3) x, $r^2 = 0.90$ at ambient and elevated CO_2 levels; and (b) y = 263 (± 5.3)−6.5 (± 0.45) x, $r^2 = 0.93$ at ambient CO_2 level and y = 250 (± 4.9)−6.5 (± 0.45) x, $r^2 = 0.93$ at elevated CO_2 level. Vertical bars denote ± SE, and are shown where they exceed the size of the symbol.

elevated CO_2, and the pod-set averaged about 67%. However, exposure to 40/30 °C, significantly ($P < 0.05$) reduced pod-set from 67 to 15% at ambient CO_2 and to 10% at elevated CO_2 level.

The percentage of flowers setting seeds was significantly affected by temperature ($P < 0.001$) and CO_2 ($P < 0.05$) treatments, but not by their interaction, therefore the response of seed-set to temperature was described by two parallel lines (Fig. 1b). As the temperature increased from 28/18 °C to 40/30 °C, seed-set was reduced by 6% per degree rise in temperature (°C^{-1}) at both ambient and elevated CO_2 levels. Based on linear regressions, the ceiling temperatures (temperature at which there was no seed-set) were 40/30 and 38/28 °C, respectively, at ambient and elevated CO_2 levels.

Pollen numbers and viability

There was no significant effect of CO_2 levels on number of pollen grains per flower and pollen viability at different temperature treatments (Fig. 2). Therefore, a single line described pollen number and pollen viability response to temperature at both ambient and elevated CO_2 levels. There was a strong negative linear relation between pollen number and temperature above a critical value of 32/22 °C. At temperatures >32/22 °C, regressed pollen number was reduced 250 per flower °$C^{-1}$and no pollen was produced in flowers at 40/30 °C.

Similar to pollen number, pollen viability at both the levels of CO_2 decreased above a critical value of 33/23 °C (Fig. 2b). As temperature increased above the critical

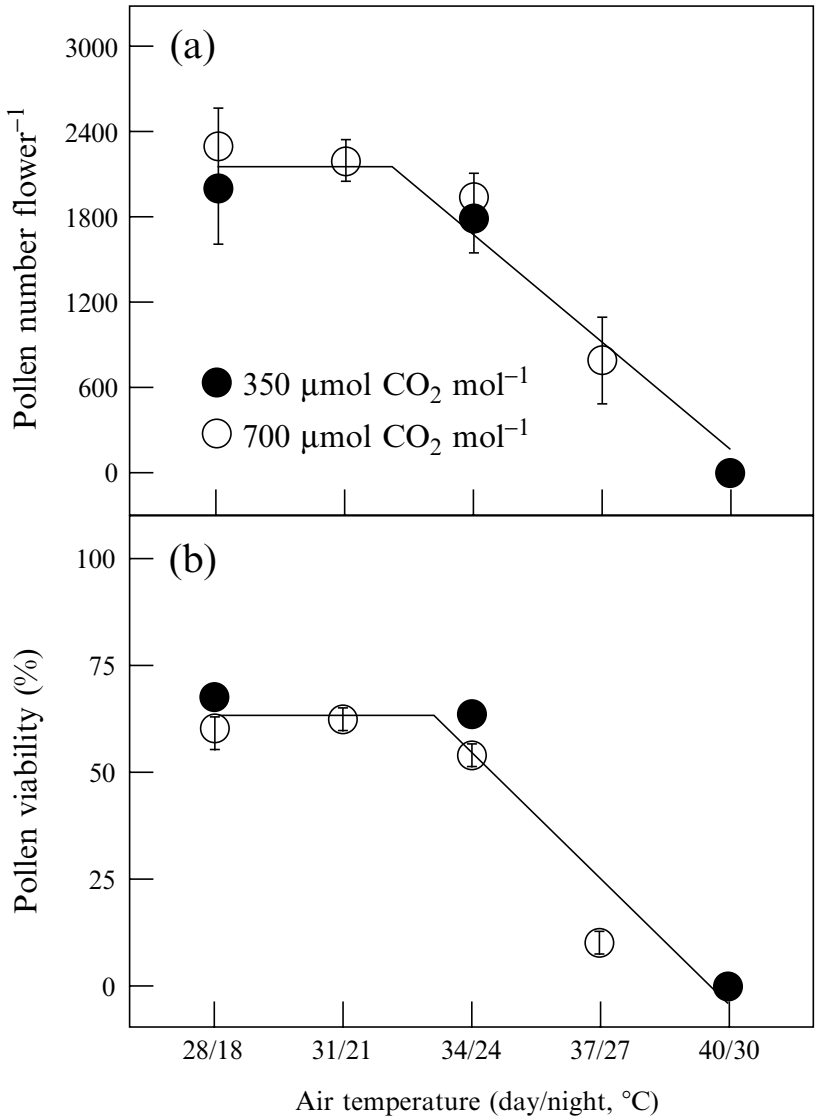

Fig. 2 Relations between day/night air temperature (°C) and (a) total number of pollen grains per flower, and (b) percentage of viable pollen grains (pollen viability, angular transformed) at ambient (●, 350 µmol mol^{-1}) and elevated (○, 700 µmol mol^{-1}) CO_2 levels. Fitted regressions for sloping lines at ambient and elevated CO_2 levels in (a) y = 10259 (± 1311)−252 (± 37) x, $r^2 = 0.94$; and (b) y = 388 (± 66)−9.8 (± 1.8) x, $r^2 = 0.91$. Vertical bars denote ± SE, and are shown where they exceed the size of the symbol.

value, viability of pollen grains was reduced by about 10% °C^{-1} under ambient and elevated CO_2 levels. However, comparing the two temperatures in common where data were not zero, overall pollen viability at elevated CO_2 level was comparatively lower (57%) than those produced at ambient CO_2 level (65%).

Seed number and yield

There were strong negative linear effects of temperature at both the CO_2 levels on seed number, seed yield and total dry weight (Fig. 3). For seed number and seed yield per plant this effect was significant for temperature, CO_2 and their interaction ($P < 0.05$). Accordingly, two lines with different slopes described the response of seed number and seed yield (Fig. 3a,b) to temperature. As temperature increased from 28/18 to 40/30 °C, the number of seeds per plant were reduced by 2.3 and 3.2 °C^{-1}, and seed yield was reduced by 1.2 g and 1.5 g plant^{-1} °C^{-1}, at ambient and elevated CO_2 levels, respectively (Fig. 3a,b). The ceiling temperature for seed number and seed yield was 37/27 °C at both ambient and elevated CO_2 level (Fig. 3). Overall, elevated CO_2 levels increased seed number from 9.8 to 12.4 plant^{-1} and seed yield from 4.6 to 5.6 g plant^{-1}. In general the absolute effects of elevated CO_2 levels on seed number, and seed yield were smaller at higher temperatures (Fig. 3).

For total dry weight, there was a significant negative effect of temperature and positive effect of CO_2 levels, but their interaction was not significant (Fig. 3c). Thus, two parallel lines with different intercepts best described the response of total dry weights to temperature (Fig. 3c). As temperature increased from 28/18 to 40/30 °C, total dry weight was reduced by about 1.6 g plant^{-1} °C^{-1} at both ambient and elevated CO_2 levels.

Seed number per pod and seed size

There were no significant effects of CO_2 on seed number per pod and individual seed weight at maturity (seed size) at different temperatures; therefore, a single line described the response to temperature at both ambient and elevated CO_2 levels. Seed number per pod was linearly reduced by 0.34 °C^{-1} above 28/18 °C (Fig. 4a). There was no effect of temperature up to 31/21 °C on seed size (Fig. 4b), but further increase in temperature, decreased seed size by 0.07 g °C^{-1} under both ambient and elevated CO_2 levels.

Photosynthesis, stomatal conductance and transpiration

There were significant ($P < 0.05$) effects of CO_2, temperature, and their interaction on leaf photosynthesis; thus, two lines with different slopes and intercepts described the response of leaf photosynthesis to temperature and CO_2 (Fig. 5a). Increase in temperature from 28/18 to 40/30 °C significantly and linearly decreased leaf photosynthesis by 0.3 μmol m^{-2}s^{-1} °C^{-1} at ambient CO_2 and by 0.9 μmol m^{-2}s^{-1} °C^{-1}at elevated CO_2 levels. Overall, elevated CO_2 increased leaf photosynthesis by 50%, i.e. from 20.2 μmol m^{-2}s^{-1} to 30.4 μmol m^{-2}s^{-1}. The beneficial effects of elevated CO_2 levels on photosynthesis decreased both absolutely and proportionately with increase in temperature, e.g. CO_2 enrichment increased leaf photosynthetic rate by 66, 43 and 39% at day/night temperature regimes of 28/18, 34/24 and 40/30 °C, respectively (Fig. 5a).

Stomatal conductance was significantly affected by carbon dioxide ($P < 0.001$), with significantly lower stomatal conductance at elevated CO_2 levels as compared to ambient CO_2 level (Fig. 5b). Although the effect of temperature on stomatal conductance was not significant, there was an increasing trend at higher temperatures as indicated in Fig. 5(b). In contrast, transpiration rates were affected by both CO_2 and temperature ($P < 0.05$). The rates of transpiration were lower at elevated CO_2, whereas, rate of transpiration increased at temperature above 28/18 and 34/24 °C at both ambient and elevated CO_2 levels (Fig. 5c).

Discussion

There were significant negative linear relations between temperature and seed-set, pollen viability, pollen number, seed number, seed yield, total dry matter production, seeds per pod, seed size, and photosynthesis, at both ambient and elevated CO_2 levels (Figs 1–5). Response of seed-set, seeds per pod, seed numbers, seed and total dry matter production and photosynthesis were well described by linear regressions over a range of temperatures from 28/18 to the warmest (ceiling) temperature in both ambient and elevated CO_2 levels. The response of pod-set, pollen numbers, pollen viability and seed size were described by two segment analysis with no effect of temperature until critical temperature value, however, above the critical temperatures pod-set (37/27 °C), pollen number (32/22 °C), pollen viability (33/23 °C) and seed size (31/21 °C) were linearly reduced. Research in controlled environments on different botanical types of bean has shown that exposure to high temperature reduced seed-set and pollen viability (Monterroso & Wien, 1990; Gross & Kigel, 1994). In our study there was no effect of temperature up to 37/27 °C on the percentage of flowers setting pods, however, the percentage of flowers setting seeds decreased linearly as temperature increased above 28/18 °C (Fig. 1) as did the seeds per pod (Fig. 4a). All the pods produced at temperatures of 37/27 and 40/30 °C

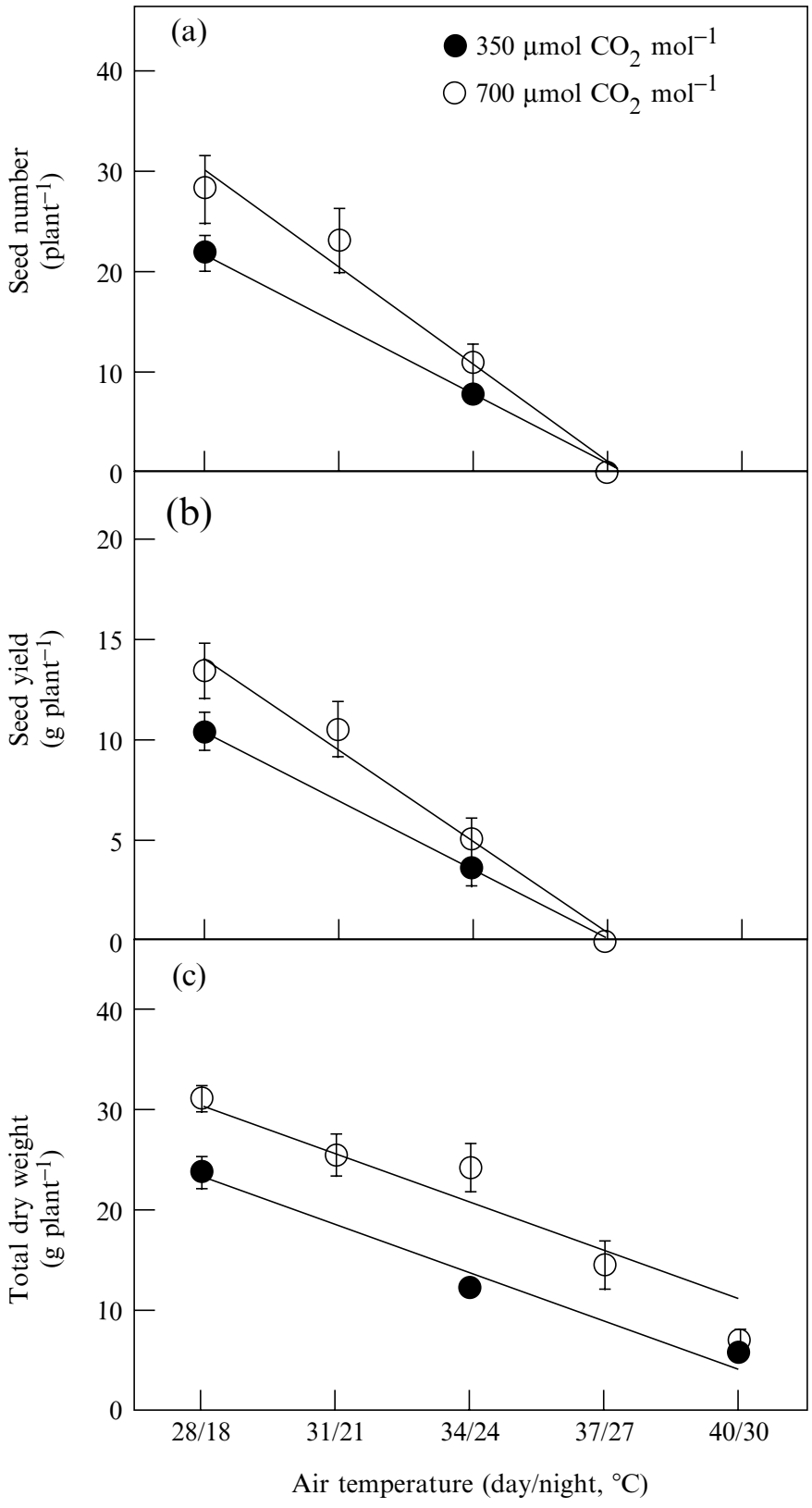

Fig. 3 Relations between day/night air temperature (°C) and (a) number of seeds per plant; (b) seed yield per plant; and (c) total dry weight per plant at ambient (●, 350 µmol mol^{-1}) and elevated (○, 700 µmol mol^{-1}) CO_2 levels. Fitted regressions for sloping lines in (a) $y = 87.2–2.3x$, $r^2 = 0.99$ at ambient CO_2 level and $y = 120.7 (\pm 11.4) – 3.2 (\pm 0.35)\, x$, $r^2 = 0.88$ at elevated CO_2 level; (b) $y = 42.5–1.2x$, $r^2 = 0.99$ at ambient CO_2 level and $y = 56.7 (\pm 4.4) – 1.5 (\pm 0.13)\, x$, $r^2 = 0.88$ at elevated CO_2 level; and (c) $y = 68.3 (\pm 6.12) – 1.6 (\pm 0.23)\, x$, $r^2 = 0.95$ at ambient CO_2 level and $y = 75.5 (\pm 8.25) – 1.6 (\pm 0.23)\, x$, $r^2 = 0.88$ at elevated CO_2 level. Vertical bars denote ±SE, and are shown where they exceed the size of the symbol.

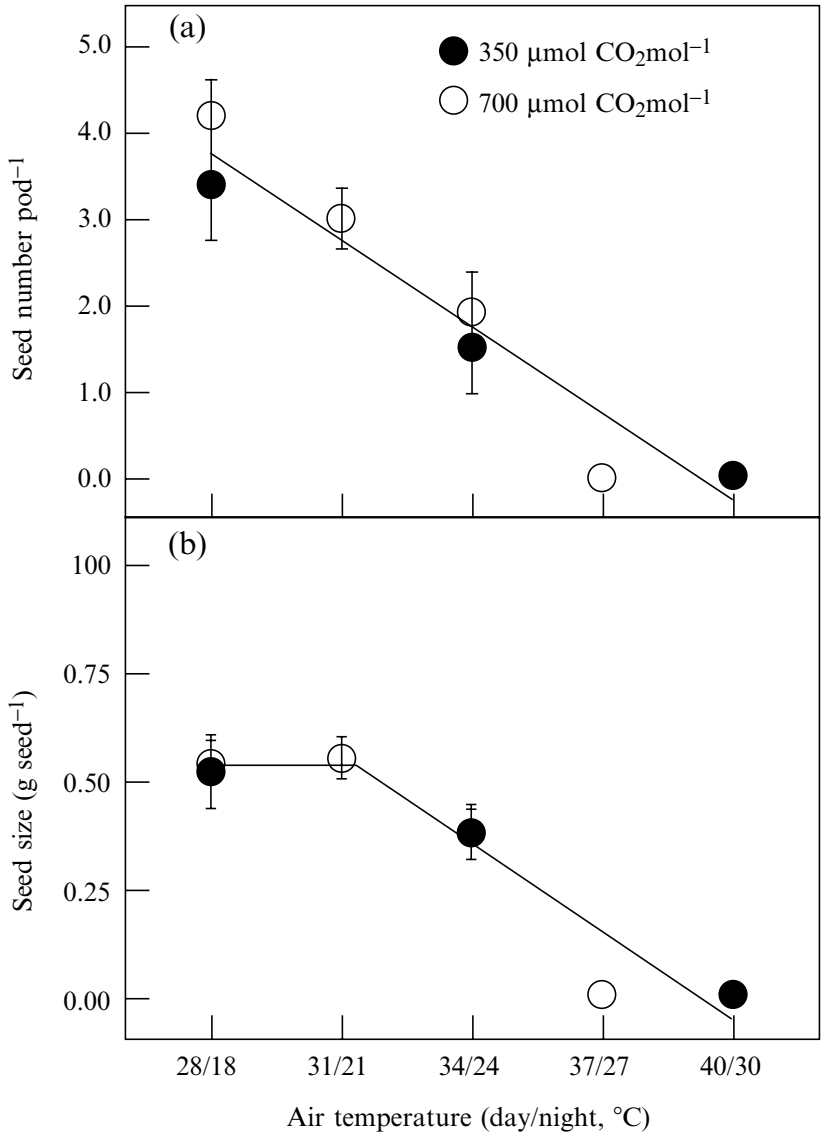

Fig. 4 Relations between day/night air temperature (°C) and (a) number of seeds per pod, and (b) weight per seed (seed size) at maturity, at ambient (●, 350 μmol mol^{-1}) and elevated (○, 700 μmol mol^{-1}) CO_2 levels. Fitted regressions for sloping lines at ambient and elevated CO_2 levels in (a) $y = 13.2\ (\pm 1.18) - 0.34\ (\pm 0.034)\ x$, $r^2 = 0.94$; and (b) $y = 2.66\ (\pm 0.55) - 0.067\ (\pm 0.015)\ x$, $r^2 = 0.86$. Vertical bars denote $\pm$SE, and are shown where they exceed the size of the symbol.

were parthenocarpic, small (< 3 cm long), sickle-shaped and did not have seeds. Furthermore, at 37/27 °C, fewer (500) pollen grains flower^{-1} were produced and of these, only 10% were viable. Therefore, the reduced seed-set at higher temperatures is likely a result of lower anther dehiscence and pollen sterility (Monterroso & Wien, 1990; Gross & Kigel, 1994). Similar effects on pollen and fruit-set have been observed in peanut (*Arachis hypogaea* L.; Prasad *et al.*, 1999, 2000, 2001), cowpea (Hall, 1992) and tomato (*Lycopersicon esculentum* Mill; Peet *et al.*, 1998). In general pollen has been reported to be more sensitive to high temperature than female reproductive structures (Monterroso & Wien, 1990); however, effects of high temperature on female fertility could not be dismissed (Gross & Kigel, 1994).

Recent study of the mechanisms of high temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of bean has shown that heat stress results in anther indehiscence, reduction in endothelial wall thickness and complete degeneration of inter locular septa in heat-susceptible genotypes (Porch & Jahn, 2001). Furthermore, Suzuki *et al*. (2001) showed that pollen sterility associated with tapetal degeneration at high temperatures in bean was mainly owing to structural abnormality and distribution of endoplasmic reticulum in tapetal cells. Furthermore, degeneration of tapetum occurred earlier under high temperature conditions than under optimum temperature conditions (Suzuki *et al*., 2001). Premature degeneration of tapetum tissue reduces nourishment to developing pollen and also affects translocation of amino acid proline from anther walls to pollen, which plays an important role in the viability or fertility of pollen grains (Mutters *et al*., 1989; Ahmed *et al*., 1992; Hesse & Hess, 1994).

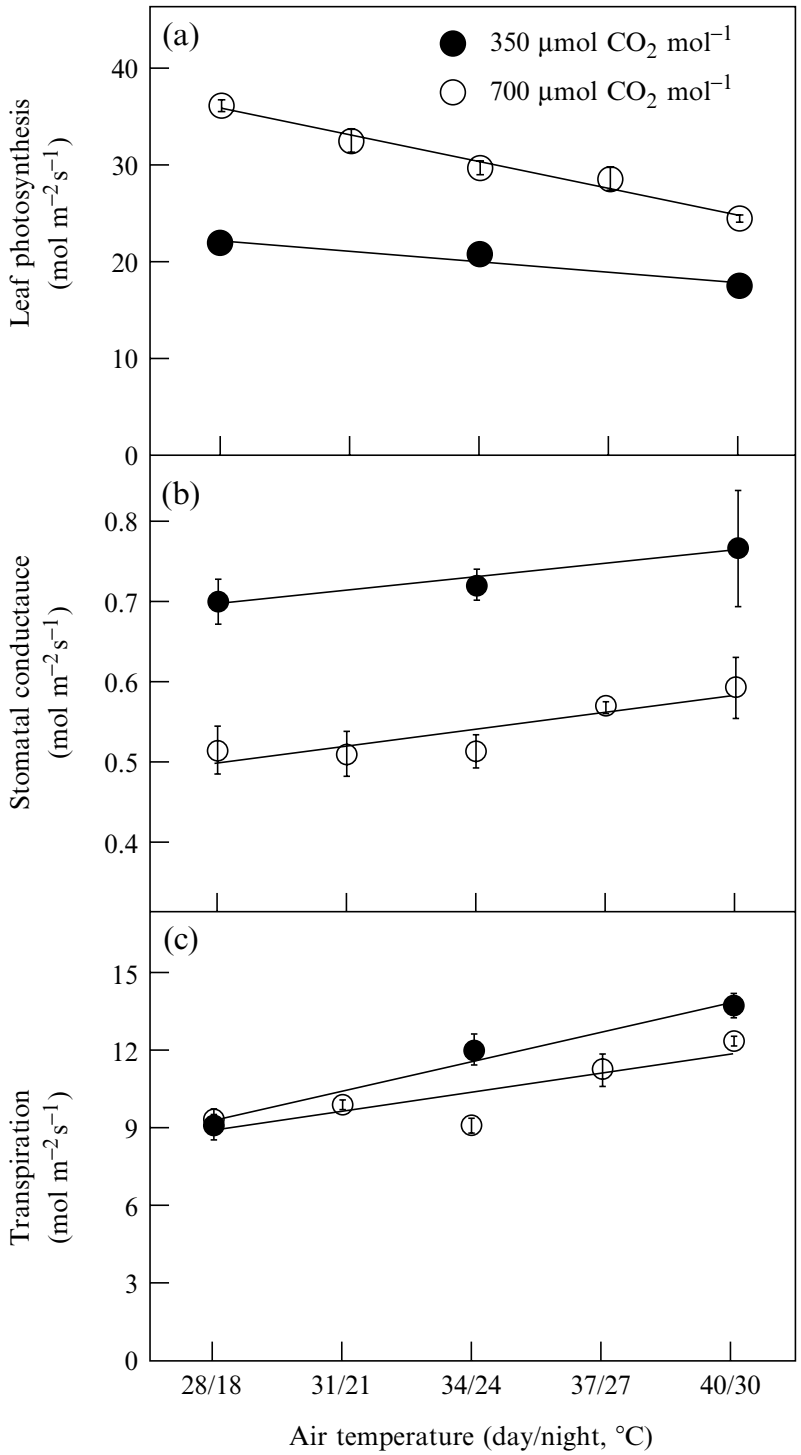

Fig. 5 Relations between day/night air temperature (°C) and rates of single-attached-leaf (a) photosynthesis; (b) stomatal conductance; and (c) transpiration at ambient (●, 350 μmol mol^{-1}) and elevated (○, 700 μmol mol^{-1}) CO_2 levels. Fitted regressions for sloping lines in (a) y = 31.6 (± 3.6)−0.34 (± 0.10) x, r^2 = 0.91 at ambient CO_2 level and y = 61.0 (± 2.8)−0.90 (± 0.08) x, r^2 = 0.98 at elevated CO_2 level; (b) y = 0.54 (± 0.04) + 0.006 (± 0.001) x, r^2 = 0.82 at ambient CO_2 level and y = 0.29 (± 0.07) + 0.006 (± 0.001) x, r^2 = 0.82 at elevated CO_2 level; and (c) y = 1.7 (± 1.0) + 0.39 (± 0.05) x, r^2 = 0.98 at ambient CO_2 level and y = 1.8 (± 1.1) + 0.25(± 0.09) x, r^2 = 0.72 at elevated CO_2 level. Vertical bars denote ± SE, and are shown where they exceed the size of the symbol.

Exposure to elevated levels of CO_2 (700 μmol mol^{-1}) increased photosynthesis, number of seeds, seed yield and total dry weight of kidney bean as typically observed in most of the food crops, including rice (*Oryza sativa* L.; Baker & Allen, 1993), soybean (Allen & Boote, 2000) and peanut (Clifford *et al*., 2000). In most, but not all C3 crops, the CO_2 level of 370 μmol mol^{-1} is a major limiting factor for the photosynthesis, growth and productivity (Bowes, 1993). Photosynthetic rates are a direct result of the activity of ribulose bisphosphate carboxylase–oxygenase (Rubisco) enzyme, which is strongly influenced by CO_2 levels. The current level of CO_2 is insufficient to saturate Rubisco in C3 species; therefore an increased availability of CO_2 results in greater leaf photosynthetic rates (Bowes, 1993) and enhances biomass accumulation. In bean, Jolliffe & Ehret (1985) observed that both pod and total dry weights linearly increased as CO_2 levels increased from 340 to 1200 μmol mol^{-1} at which maximum increase was observed, and further enrichment to 2000 or 3000 μmol mol^{-1} had no additional effect.

There was no significant effect of CO_2 levels on the percentage of flowers setting pods, and number of pollen grains per flower, but the percentage of flowers setting seeds was significantly lower at elevated CO_2 in spite of greater photosynthesis rates (Figs 1, 2 and 5). Individual seed weight at maturity and number of seeds per pod was not affected by elevated CO_2 (Fig. 4). Greater yields at elevated CO_2 were mainly owing to greater total number of seeds at maturity (Fig. 3). This clearly suggests that reduced seed-set was not a result of reduced availability of photosynthates. Similar observation was made on soybean where growth rate of individual seeds and seed size were not affected by CO_2 over the range of 330–990 μmol CO_2 mol^{-1} (Allen *et al*., 1991). The lower seed-set at elevated CO_2 levels may be a result of greater number of flowers produced owing to improved vegetative growth and branching (Jolliffe & Ehret, 1985) or small shift in tissue temperature at elevated CO_2. Elevated CO_2 reduced the ceiling temperature for seed-set by about 2 °C compared to those at ambient CO_2 levels (Fig. 1b). Similarly in rice, Matsui *et al*. (1997) observed that the critical temperature for spikelet sterility (as determined from the number of germinated pollen grains on the stigma) was reduced by 1 °C at elevated (660 μmol mol^{-1}) concentrations of CO_2. Our findings and that of Matsui *et al*. (1997) suggest that the elevated CO_2 increased pollen susceptibility to high temperature by 1–2 °C. The exact mechanism through which elevated CO_2 increases susceptibility to high temperature via increased pollen sterility needs further investigation. However, one possibility is small increase in tissue temperatures under CO_2 enrichment. In our study, the leaf temperature of plants grown at elevated CO_2 levels was about 1.5 °C higher than those grown at ambient CO_2, across the 28–40 °C ranges of mid-day chamber air temperatures (Fig. 6). Using similar experimental chambers, Pan (1996) reported that bulk foliage temperature of soybean were 1–2 °C greater at elevated CO_2 levels. This phenomenon occurs because elevated CO_2 causes partial closure of stomata and thereby increases leaf resistance to water vapour efflux, resulting in decreased transpiration rate, as supported by data shown in Fig. 5. Decreased transpiration causes leaves to be warmed slightly because less latent heat is lost (Allen *et al*., 1985). Energy balance simulations with soil–plant-atmospheric models (Allen, 1990; Boote *et al*., 1997) also show that foliar temperatures increased about 1 °C with doubling of CO_2 concentration, owing to decreased leaf conduc-

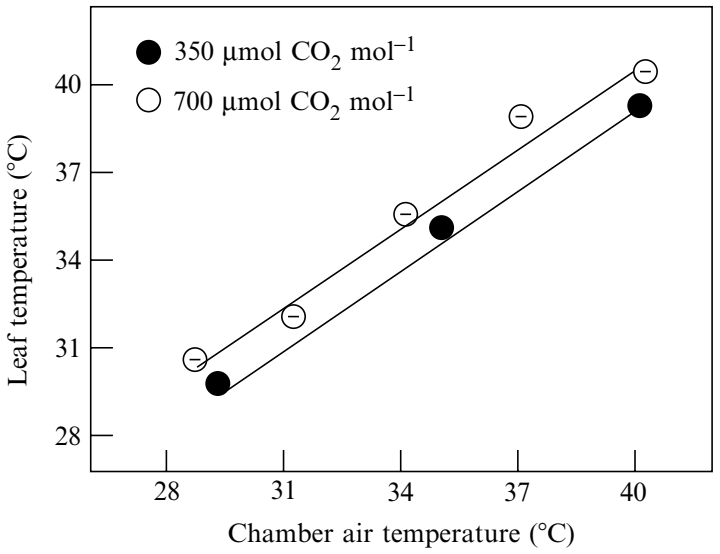

Fig. 6 Relations between controlled-environment chamber air temperature (°C) using thermocouples and leaf temperatures measured using the LI-COR photosynthetic system at ambient (●, 350 μmol mol^{-1}) and elevated (○, 700 μmol mol^{-1}) CO_2 levels. Measurements were made between 1200 and 1400 h when photosynthetic photon irradiance ranged between 1700 and 1900 μmol $m^{-1}s^{-1}$. Fitted regression for sloping lines at ambient and elevated CO_2 levels: $y = 4.44\ (\pm 1.67) + 0.90(\pm 0.05)\ x$, $r^2 = 0.99$. Vertical bars denote ± SE, and are shown where they exceed the size of the symbol.

tance. While flower temperature is rarely measured, one could speculate that flower temperature in a bean canopy should reflect bulk canopy temperature response to elevated CO_2.

This research has clearly shown that there was no beneficial interaction of elevated CO_2 with higher temperature in bean. At both the levels of CO_2, higher temperatures resulted in significant yield losses. The beneficial effects of elevated CO_2 levels on photosynthesis and growth, were overwhelmed by the negative effects of high temperatures on reproductive growth (pod-set, seed-set, pollen number, pollen viability and pod yield) in bean (Figs 1–5). Furthermore, the fact that the ceiling temperature for seed-set was 2 °C lower at elevated CO_2 suggests that yield losses associated with high temperature will increase with elevated CO_2. This is similar to the findings on other plant species, such as rice (Baker & Allen, 1993), soybean (Baker *et al*., 1989), cowpea (Ahmed *et al*., 1993), and cotton (*Gossypium hirsutum* L.; Reddy *et al.,* 1995), where high temperatures negated effects of elevated CO_2. Thus, if climate change is associated with increased temperature, economic yield of crops that are sensitive to high temperature during the reproductive phase will be reduced even after taking account of the beneficial effects of CO_2 enrichment. Research has shown that heat tolerant cultivars of cowpea are more responsive to elevated CO_2 with respect to seed production under both high and intermediate temperatures (Ahmed *et al*., 1993) suggesting that heat tolerance may be an important criterion in breeding cultivars that can adapt to the future climates. Certain genotypes of bean have better seed-set and pollen viability at higher temperature (Agtunong *et al*., 1992; Gross & Kigel, 1994; Porch & Jahn, 2001). Therefore, a global search for genetic materials that are more tolerant to high temperatures for seed production is needed for bean and other seed crops to improve productivity in present and future climates. In addition, it may also be useful to examine if stomatal sensitivity to CO_2 varies among cultivars, and attempts should be made to identify those cultivars that have lower stomatal sensitivity to elevated CO_2.

Acknowledgements

We acknowledge the support of Agronomy Department, University of Florida and United States Department of Agriculture, Agriculture Research Service. This work is a contribution of the University of Florida and approved for publication as Florida Agricultural Experiment Station Journal Series No. R-08515. We thank Wayne Wynn and Andy Frenock for engineering support.

References

Agtunong TP, Redden R, Mengge-Nang MA, Searle C, Fukai S (1992) Genotypic variation in response to high temperature at flowering in common bean (*Phaseolus vulgaris* L.). *Australian Journal of Experimental Agriculture*, **32**, 1135–1140.

Ahmed FE, Hall AE, Demason DA (1992) Heat injury during floral development in cowpea (*Vigna unguiculata*, Fabaceae). *American Journal of Botany*, **79**, 784–791.

Ahmed FE, Hall AE, Madore MA (1993) Interactive effects of high-temperature and elevated carbon dioxide concentration on cowpea (*Vigna unguiculata* L. Walp). *Plant Cell and Environment*, **16**, 835–842.

Allen LH Jr (1990) Plant responses to rising carbon dioxide and potential interactions with air pollutants. *Journal of Environmental Quality*, **19**, 14–34.

Allen LH Jr, Bisbal EC, Boote KJ, Jones PH (1991) Soybean dry matter allocation under subambient and superambient levels of carbon dioxide. *Agronomy Journal*, **83**, 875–883.

Allen LH Jr, Boote KJ (2000) Crop ecosystem responses to climate change: soybean. In: *Climate Change and Global Crop Productivity* (eds Reddy KR, Hodges HF), pp. 133–160. CAB International, Wallingford, Oxon, UK.

Allen LH Jr, Jones PH, Jones JW (1985) Rising atmospheric CO_2 and evapotranspiration. In: *Advances in Evapotranspiration.* Proceedings of National Conference on Advances in Evapotranspiration, pp. 13–27. ASAE Publication 14–85. American Society of Agricultural Engineers, St Joseph, Michigan, USA.

Baker JT, Allen LH Jr (1993) Effects of CO_2 and temperature on rice: summary of five growing seasons. *Agricultural and Forest Meteorology*, **48**, 575–582.

Baker JT, Allen LH Jr, Boote KJ, Jones P, Jones JW (1989) Response of soybean to air temperature and carbon dioxide concentration. *Crop Science*, **29**, 98–105.

Boote KJ, Pickering NB, Allen LH Jr (1997) Plant Modeling: Advances and gaps in our capability to project future crop growth and yield in response to global climate change. In: *Advances in Carbon Dioxide Effects Research* (eds Allen LH Jr, Kirkham MB, Olszyk DM, Whitman CE), pp. 179–228. ASA Special Publication No: 61, ASA-CSSA-SSSA, Madison, WI, USA.

Bowes G (1993) Facing the inevitable: Plant and increasing atmospheric CO_2. *Annual Review of Plant Physiology and Plant Molecular Biology*, **44**, 309–332.

Clifford SC, Stronach IM, Black CR, Singleton-Jones PR, Azam Ali SN, Crout NMJ (2000) Effects of elevated CO_2, drought and temperature on the water relations and gas exchange of groundnut (*Arachis hypogaea*) stands grown in controlled environment glasshouses. *Physiologia Plantarum*, **110**, 78–88.

Davis JHC (1997) *Phaseolus* Beans. In: *The Physiology of Vegetable Crops* (ed. Wien HC), pp. 409–428. CAB International, Wallingford, Oxon, UK.

Gross Y, Kigel J (1994) Differential sensitivity to high temperature of stages in the reproduction development of common beans (*Phaseolus vulgaris* L.). *Field Crops Research*, **36**, 201–212.

Hall AE (1992) Breeding for heat tolerance. *Plant Breeding Reviews*, **10**, 129–168.

Hesse M, Hess M (1994) Recent trends in tapetum research – a cytological and methodological review. *Plant Systematics and Evolution,* [(Suppl.)], **7**, 127–145.

IPCC (2001) *Climate Change 2001. The scientific basis*. Contribution of working group I to the third assessment report of the intergovernmental panel on climate change (eds. Ding *et al.*). Cambridge University Press, Cambridge, p892.

Jolliffe PA, Ehret DL (1985) Growth of bean plants at elevated carbon dioxide concentrations. *Canadian Journal of Botany*, **63**, 2021–2025.

Kearns CA, Inouye DW (1993) *Techniques for Pollination Biologists*. University Press, Colorado, p583.

Konsens I, Ofir M, Kigel J (1991) The effect of temperature on the production and abscission of flowers and pod in snap bean (*Phaseolus vulgaris* L.). *Annals of Botany*, **67**, 391–399.

Matsui T, Namuco T, Ziska LH, Horie T (1997) Effects of high temperature and CO_2 concentration on spikelet sterility in indica rice. *Field Crops Research*, **51**, 213–219.

Mead R, Curnow RN, Hasted AM (1993) *Statistical Methods in Agriculture and Experimental Biology*. Champan and Hall, London, p415.

Monterroso VA, Wien HC (1990) Flower and pod abscission due to heat stress in beans. *Journal of American Society for Horticultural Science*, **115**, 631–634.

Mutters RG, Ferreira LGR, Hall AE (1989) Proline content of the anthers and pollen of heat-tolerant and heat-sensitive cowpea subjected to different temperatures. *Crop Science*, **29**, 1497–1500.

Pan D (1996) *Soybean Responses to Elevated Temperature and Doubled CO_2*. PhD Thesis, University of Florida, Gainesville, USA.

Peet MM, Sato S, Gardner RG (1998) Comparing heat stress effects on male-fertile and male-sterile tomatoes. *Plant Cell and Environment*, **21**, 225–231.

Pickering NB, Allen LH, Albrecht SL, Jones P, Jones JW, Baker JT (1994) Environmental plant chambers: Control and measurement using CR-10T data loggers. In: *Computers in Agriculture.* Proceedings of the 5th International Conference, *Orlando FL, 5–9 February* (eds Watson DG, Zazueta FS, Harrison TV), pp. 29–35. American Society of Agricultural Engineers, St Joseph, Michigan, USA.

Porch TG, Jahn M (2001) Effect of high-temperature stress on microsporogenesis in heat-sensitive and heat-tolerant genotypes of *Phaseolus vulgaris*. *Plant Cell and Environment*, **24**, 723–731.

Prasad PVV, Craufurd PQ, Kakani VG, Wheeler TR, Boote KJ (2001) Influence of high temperature during pre-and post-anthesis stages of floral development on fruit-set and pollen germination in peanut. *Australian Journal of Plant Physiology*, **28**, 233–240.

Prasad PVV, Craufurd PQ, Summerfield RJ (1999) Fruit number in relation to pollen production and viability in groundnut exposed to short episodes of heat stress. *Annals of Botany*, **84**, 381–386.

Prasad PVV, Craufurd PQ, Summerfield RJ (2000) Effects of short episodes of heat stress on flower production and fruit-set of groundnut (*Arachis hypogaea* L.). *Journal of Experimental Botany*, **51**, 777–784.

Reddy KR, Hodges HF, Mckinion JM (1995) Carbon dioxide and temperature effects on pima cotton growth. *Agriculture Ecosystems and Environment*, **54**, 17–29.

Rosenzweig C, Hillel D (1998) *Climate Change and the Global Harvest: Potential Impacts of the Greenhouse Effect of Agriculture*. Oxford University Press Inc, New York, p324.

Suzuki K, Takeda H, Tsukaguchi T, Egawa Y (2001) Ultrastructural study on degeneration of tapetum in anther of snap bean (*Phaseolus vulgaris* L.) under heat-stress. *Sexual Plant Reproduction*, **13**, 293–299.